

Appl. No. 09/167,088

Amdt. dated Friday, June 20, 2003

Reply to Office Action of March 11, 2003

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claim 1. (currently amended) A method of measuring the production of a secreted target analyte of interest in a human or animal, comprising the steps of:

- a. injecting the human or animal with an amount of labeled neutralizing targeting moiety, wherein the targeting moiety binds specifically to the target analyte, and wherein the targeting moiety is injected in sufficient quantity that a measurable fraction of target analyte is bound by the labeled neutralizing targeting moiety;
- b. allowing the targeting moiety to circulate through the injected human or animal for a defined period of time sufficient to bind to the target analyte of interest and form a targeting moiety:target analyte conjugate wherein the formation of the targeting moiety:target analyte conjugate decreases the clearing rate of the target analyte;
- c. obtaining a sample of blood from the human or animal after the defined period of time;
- d. combining the sample of blood with a capture moiety wherein the capture moiety binds specifically to the targeting moiety:target analyte conjugate in order to form an assay mixture;
- e. incubating the assay mixture of step d to allow the capture moiety to bind to the targeting moiety:target analyte conjugate and form targeting moiety:target analyte:capture moiety complexes in the assay mixture;
- f. removing any unbound and unconjugated targeting moiety and target analyte from the assay mixture;

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- g. detecting the amount of labeled targeting moiety:target analyte:capture moiety complexes;
- h. wherein the amount of labeled targeting moiety:target analyte:capture moiety complexes detected in step (g) provides a measure of the production of secreted target analyte in the sample during the defined period of time; and
- i. wherein the secreted target analyte is a secreted cytokine, ~~or~~ secreted peptide or secreted protein hormone.

Claim 2 (cancelled)

Claim 3 (cancelled)

Claim 4. (previously amended) The method of claim 14, wherein the target analyte is a cytokine.

Claim 5. (original) The method of claim 4, wherein the cytokine is selected from the group consisting of interleukins, interferons chemokines, growth factors, colony stimulating factors, lymphokines, lymphotoxins, and tumor necrosis factors.

Claim 6. (previously amended) The method of claim 4, wherein the cytokine is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, interleukin-17, interleukin-18,, interferon-alpha, interferon-beta, interferon-gamma, lymphotoxin, tumor necrosis factor-alpha, transforming growth factor (TGF)-beta, granulocyte macrophage-colony stimulating factor (GM-CSF), nerve growth factor (NGF), and epidermal growth factor (EGF).

Claim 7. (previously amended) The method of claim 1, wherein the blood is selected from the group consisting of whole blood, serum and plasma.

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Claim 8. (previously amended) The method of claim 1, wherein the targeting moiety is selected from the group consisting of antibodies, soluble receptors, and recombinant molecules with binding sites for the target analyte.

Claim 9. (previously amended) The method of claim 8, wherein the targeting moiety is a monoclonal antibody.

Claim 10. (original) The method of claim 1, wherein the capture moiety is an antibody.

Claim 11. (original) The method of claim 10, wherein the antibody is a polyclonal antibody which recognizes many epitopes on the target analyte.

Claim 12. (previously amended) The method of claim 9, wherein the targeting moiety is detectably labeled, wherein the label is selected from the group consisting of radioisotopes, affinity labels, enzymatic labels, and fluorescent labels.

Claim 13. (previously amended) The method of claim 1, wherein the targeting moiety is labeled with a small molecule hapten and wherein the method further comprises the step of binding the small molecule hapten to a binding partner which is conjugated to an enzyme.

Claim 14. (previously amended) The method of claim 1, wherein the defined period of time is from about 1 hour to about 72 hours.

Claim 15. (previously amended) The method of claim 13, wherein the hapten is biotin.

Claim 16. (original) The method of claim 13, wherein the enzyme-conjugated binding partner is selected from the group consisting of streptavidin, anti-biotin antibody, anti-hapten antibody, and anti-immunoglobulin antibody.

Claim 17. (original) The method of claim 13, wherein the enzyme is selected from the group consisting of alkaline phosphatase, glucose oxidase, beta-galactosidase, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase,

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horseradish peroxidase, asparaginase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

Claim 18. (original) The method of claim 12, wherein the targeting moiety is labeled by linking to a fluorescent labeling compound.

Claim 19. (original) The method of claim 18, wherein the fluorescent labeling compound is selected from the group consisting of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Claim 20. (currently amended) The method of claim 8, ~~further comprising after step (a) the step of injecting the human or animal with an amount of second targeting moiety, wherein the second targeting moiety binds specifically to the first targeting moiety, wherein the second targeting moiety is injected in sufficient quantity that a measurable fraction of first targeting moiety is bound by the second targeting moiety and wherein the second targeting moiety is specifically bound by the capture moiety.~~ wherein the labeled targeting moiety comprises first and second members of a complimentary ligand/anti-ligand pair, wherein the first member of the complimentary ligand/anti-ligand pair is injected as the targeting moiety in step (a); wherein the second member of the complimentary ligand/anti-ligand pair is a detectable binding partner to the first member; and wherein the method further comprises the steps of (I) contacting the assay mixture after step (e) and before step (f) with the second member of the complimentary ligand/anti-ligand pair to allow binding of the first and second members; (II) removing any unbound second member; (III) detecting the amount of bound second member; and (IV) correlating the detected amount to the amount of targeting moiety:target analyte:capture moiety complexes in the assay mixture; wherein the amount of targeting moiety:target analyte:capture moiety complexes detected provides a measure of the production of secreted target analyte during the defined period of time.

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Claim 21. (currently amended) The method of claim 20, wherein the first member of the complimentary ligand/anti-ligand pair ~~first targeting moiety~~ is a monoclonal antibody.

Claim 22. (currently amended) The method of claim 21, wherein the ~~second targeting~~ capture moiety is an antibody.

Claim 23. (currently amended) The method of claim 22, wherein the ~~second targeting~~ capture moiety is a polyclonal antibody.

Claim 24. (cancelled)

Claim 25. (currently amended) The method of claim 20, wherein the ~~means for detecting the targeting moiety:target analyte:capture moiety complexes is~~ are detected by radioimmunoassay.

Claim 26. (currently amended) The method of claim 20, wherein the second member of the complimentary ligand/anti-ligand pair ~~second targeting moiety~~ is detectably labeled by an enzymatic label.

Claim 27. (original) The method of claim 26, wherein the label is a small molecule hapten.

Claim 28. (original) The method of claim 27, wherein the hapten is biotin.

Claim 29. (currently amended) The method of claim 26, wherein the ~~enzyme-conjugated binding partner~~ second member of the complimentary ligand/anti-ligand pair is selected from the group consisting of streptavidin, anti- biotin antibody, anti-hapten antibody, and anti-immunoglobulin antibody.

Claim 30. (original) The method of claim 26, wherein the enzyme is selected from the group consisting of alkaline phosphatase, glucose oxidase, beta-galactosidase, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase,

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horseradish peroxidase, asparaginase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

Claim 31. (currently amended) The method of claim 20, wherein the ~~capture moiety~~ second member of the complimentary ligand/anti-ligand pair is labeled with a fluorescent label ~~by linking to a fluorescent labeling compound.~~

Claim 32. (original) The method of claim 31, wherein the fluorescent labeling compound is selected from the group consisting of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Claim 33. (currently amended) The method of claim 1 or 20, wherein the capture moiety is immobilized on a solid phase support.

Claim 34. (currently amended) A reagent kit useful in measuring the production of a secreted target analyte of interest in a human or animal ~~performing the method of claim 1,~~ comprising

- (a) a first reagent comprising a labeled targeting moiety specific for the target analyte wherein the label is ~~an enzyme-indicating means~~ operatively linked to the targeting moiety;
- (b) a second reagent separated from said first reagent, wherein the second reagent comprises a capture moiety specific for binding the target analyte ~~even when such target analyte is conjugated with the labeled targeting moiety~~ at a binding site different from that for the labeled targeting moiety so that the binding of the analyte by the capture moiety may form a labeled targeting moiety:target analyte:capture moiety complex; and
- (c) a third reagent separated from said first and second reagents which contains a standard for the analyte.

Claim 35. (original) The reagent kit of claim 34, wherein the targeting moiety is an antibody.

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Claim 36. (original) The reagent kit of claim 34, wherein the capture moiety is an antibody.

Claim 37. (currently amended) A reagent kit useful in measuring the production of a secreted target analyte of interest in a human or animal performing the method of claim 20, comprising:

- (a) a ~~first container having~~ reagent comprising first targeting moieties comprising paratopic molecules that immunoreact with a target analyte, and are operatively linked to a label;
- (b) a second ~~container having~~ reagent comprising second targeting moieties comprising paratopic molecules that immunoreact with the label of the first targeting moieties ~~target analyte at a site different from the first targeting moieties but are not in the first container;~~
- (c) a ~~second~~ third reagent comprising ~~separated from said first reagent, wherein the second reagent comprises~~ a capture moiety specific for the target analyte wherein the capture moiety is specific for a determinant site on the analyte different from the determinant site recognized by the targeting moiety even when such target analyte is conjugated with the labeled targeting moiety; and
- (d) one or more other containers comprising one or more of the following: a sample reservoir, a solid phase support, wash reagents, reagents for detecting the presence of the first targeting moieties ~~from the second container,~~ or reagents for amplifying the label.

Claim 38. (previously amended) A reagent kit of claim 37, wherein the label is selected from the group consisting of radioisotopes, affinity labels, enzymatic labels, and fluorescent labels.

Claim 39. (previously amended) A reagent kit of claim 38, wherein the fluorescent labels are fluorochromes selected from the group consisting of fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride

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(DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200
and sulphonyl chloride (RB 200 SC).

Claim 40. (original) A reagent kit of claim 35, wherein the antibodies are polyclonal.

Claim 41. (original) A reagent kit of claim 35, wherein the antibodies are monoclonal.

Claim 42. (original) A reagent kit of claim 36, wherein the antibodies are immobilized on a solid
support.